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HEPATITIS B (JK LIM, SECTION EDITOR)

Diagnosis and Management of Occult Hepatitis B Infection

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Abstract

Purpose of Review Occult hepatitis B infection refers to the presence of replication-competent hepatitis B virus despite the lack of hepatitis B surface antigen due to epigenetic or immune control. Risk factors for occult hepatitis B infection include sex work, blood transfusion, hemodialysis, and liver transplantation. Historically, the recognition of occult hepatitis B infection has been important in preventing reactivation of hepatitis B infection and mortality in those receiving treatment for HIV and hepatitis C virus along with those receiving immunosuppressive therapy.

Recent Findings Recognition of occult hepatitis B infection has increased and continues to evolve with improved detection methods in both serum and liver tissue to identify HBV DNA. Advancements in the understanding of genetic variations of hepatitis B have also contributed to the differentiation of overt versus occult hepatitis B infection. More recent research has contributed to the debate that occult hepatitis B infection may play a role in clinical outcomes such as chronic liver disease and hepatocellular carcinoma.

Summary With enhanced awareness of occult hepatitis B and improved methods for detection of HBV DNA and hepatitis B surface antigen, future studies understanding the natural history will shape policy regarding therapeutics and preventive strategies to prevent occult hepatitis B–related morbidity and mortality.

Keywords Occult hepatitis B virus infection

Introduction

Occult hepatitis B virus (HBV) infection (OBI) is defined by the presence of replication-competent HBV DNA in the liver and/or blood despite a negative hepatitis B surface antigen (HBsAg) by currently available assays [1••]. OBI prevalence has been difficult to estimate due to a lack of standardized testing and variation in sensitivity of assays, populations studied, and the number of blood samples tested over time. Despite these limitations, OBI appears to be most prevalent in developing countries, especially in areas where hepatitis B is endemic [1••]. Recently, it has been demonstrated that the global impact of OBI is increasing due to the prevalence of OBI in immigrants in developed countries [2].

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As evolving research has improved detection and increased awareness of its potential role in clinical outcomes, the significant effect of OBI must be acknowledged in the development of healthcare policy at both preventive and therapeutic levels. International experts have convened in Taormina, Italy, most recently in 2018, to continue to provide insight into ongoing research in OBI [1••]. The purpose of this review is to increase awareness of OBI among providers and researchers and to highlight recent advancements in this field.

Defining Occult Hepatitis B: True or False?

Diagnosis of Occult Hepatitis B Infection

Replication-competent HBV DNA can be measured intrahepatically via liver tissue and/or in blood. While the activity of HBV DNA in OBI is low, often resulting in intermittently low or no levels of viremia, it retains its replication and transcription properties. Categorization of OBI depends on the serologic antibody profile of hepatitis B core antibody (HBcAb) and hepatitis B surface antibody (HBsAb) (Fig. 1). Seropositive OBI is defined by the presence of HBcAb and/or HBsAb.

Seronegative OBI is defined by the absence of both serologic HBcAb and HBsAb. Identification of seronegative OBI can be difficult and leads to delays in the diagnosis and prevention of HBV reactivation. A summary of different scenarios by serologic and virologic evaluation for OBI is shown in Table 1.

As the verification of OBI can be variable across studies, it is important to define replication-competent HBV DNA as the presence of episomal cccDNA, the primary resource for viral replication. The detection of this in liver tissue is considered the gold standard for diagnosis of OBI [1••]. Serum detection of HBcAb has been used as a surrogate measure to identify cases of OBI [3]. In cases of isolated HBcAb positivity and negative serum HBV DNA, further investigation is usually necessary to identify intrahepatic cccDNA for a diagnosis of OBI. However, this would obligate each patient to invasive methods for obtaining liver tissue which may be an unwelcome measure in the setting of asymptomatic or lack of OBI disease. Of note, conventional methods of assessing intrahepatic HBV DNA can also identify integrated HBV DNA fragments, which are considered replication deficient due to incomplete genome. A study by Caviglia et al. used droplet digital PCR and identified intrahepatic cccDNA in only 52% of those classically diagnosed with OBI [4•]. Furthermore, the authors utilized the serum IgG antibody to hepatitis B core antigen to infer the presence of intrahepatic cccDNA. Future studies in OBI evaluating non-invasive methods to identify intrahepatic cccDNA are warranted.

Seroclearance of Hepatitis B Surface Antigen

In seropositive OBI, cases can arise as two scenarios: (1) the clearance of an acute self-limiting HBV infection or (2) the seroclearance of HBsAg after chronic infection. In the first scenario, OBI is often seen in cases of concomitant liver diseases, cryptogenic cirrhosis, or hepatocellular carcinoma (HCC) who had previously been infected and cleared. In the second case, HBsAg seroclearance is often noted in patients with a previous history of uncomplicated chronic HBV who have been followed serially while on or off treatment. In cases of HBsAg seroclearance, HBV DNA may still exist in the liver, but the replication potential is considered suppressed due to the host immune response. However, despite the presence of replication-defective HBV DNA, it is known that individuals with HBsAg seroclearance have a reduced but persistent risk of HCC [5].

Genetic Variations and HBsAg in "Occult" Hepatitis B Infection

The lack of detection of HBsAg may be related to genetic variances leading to a modified HBsAg that "escapes" conventional assays. Pre-S1/S2/S gene mutations have been implicated in OBI [6, 7]. This entity is often referred to as "false" OBI given serum HBV DNA levels are often equivalent to that in overt cases of HBV [8]. Several newer mutations of the S region have been recently discovered [9], including an E2G mutation which limits the mechanism of HBsAg secretion to the extracellular space.

Differentiating overt from occult HBV is determined primarily by the detection of HBsAg. Recent advancements have been made in increasing the sensitivity of HBsAg, such as using newer chemiluminescent enzyme immunoassay for detection such as that used in Japan [10] and Spain [11]. The implementation of newer HBsAg assays will certainly change

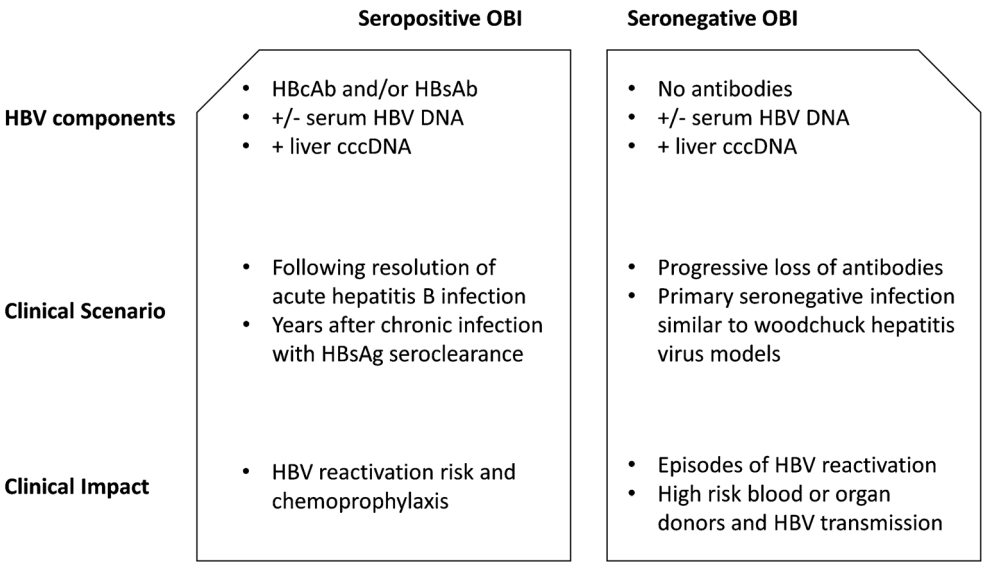

Fig. 1 Serologic profile of occult hepatitis B infection. Abbreviations: HBcAb = hepatitis B core antibody; HBsAb = hepatitis B surface antibody; HBsAg = hepatitis B surface antigen; HBV = hepatitis B virus; OBI = occult hepatitis B infection

Table 1 Different serologic and virologic scenarios of occult hepatitis B infection (OBI)

	Serum HBcAb	Serum HBsAb	Serum HBV DNA	Intrahepatic HBV DNA
Not infected	−	−	−	−
Vaccinated	−	+	−	−
Seropositive OBI	+	+/−	+	+
Seropositive OBI with undetectable viremia*	+	+/−	−	+
Seronegative OBI	−	−	+	+
Seronegative OBI with undetectable viremia*	−	−	−	+
Chronic hepatitis B with genetic variation	+	−	+	+
HBcAb+ donated liver	+	+/−	+/−	+
High-risk donated liver	−	+/−	−	+

*HBV DNA levels too low to be detected by conventional assays

the diagnosis of OBI to overt HBV. Newer diagnostic studies for HBV components are displayed in Table 2.

Risk Factors for OBI

Risk factors for OBI follow similarly to those in HBV transmission. Differences in antiviral T and B cell response in HBV control are what differentiate the development of OBI from chronic overt HBV infection.

Sex Workers

Sex worker groups, including heterosexual women and men who have sex with men, are at high risk of blood-borne viral transmissions, including OBI. A previous study evaluating female sex workers in Turkey reported a prevalence rate of 4.5% [17]. A recent study from Kenya reported a higher prevalence rate of 11.2% of OBI out of 89 male sex workers who primarily have sex with men [18]. Screening and vaccination measures are important to pursue in high-risk cohorts, such as sex workers, to decrease the risk of HBV transmission.

Blood Transfusion

Blood transfusion is a major risk factor in transmitting HBV from donors with OBI. Given the limitations of current diagnostics, the screening of blood donors can occasionally miss OBI. Although the likelihood of transmission by blood transfusion has previously been reported to be low [19], the prevalence may be underestimated due to the lack of universal HBV testing, limitations in current testing of donors, and the lack of pre-transfusion sampling in recipients to evaluate for pre-existing HBV infection. Transfusion risk is greatest in developing countries due to the lack of HBcAb and/or HBV DNA testing during the screening of blood donors. Developed countries utilize nucleic acid testing, which has improved sensitivity in identifying HBV and hence OBI compared to only serologic HBsAg detection [20]. However, there is still a persistent risk of transmission as the minimal HBV DNA threshold level leading to infection can remain below the detection limit of conventional assays [1]. The infectivity difference of seropositive vs seronegative OBI is unknown. However, donors with OBI who are HBsAb-positive may have the lowest risk of HBV transmission [19].

Table 2 New diagnostic tests for occult hepatitis B infections

Study	HBV component	Tissue or serum	Type of assay
Akram et al. JVH 2018 [12]	HBV DNA	Serum	Loop-mediated isothermal amplification
Caviglia et al. J Hepatol 2018 [4•]*	HBV cccDNA	Tissue	Droplet digital PCR
Saitta et al. Liver Int 2015 [13] Wong et al. CGH 2020 [14••]	Integrated HBV DNA	Tissue	Alu-PCR
Liu et al. J Hepatol 2019 [15]	HBV cccDNA and rcDNA	Tissue	Over-gap PCR amplification
Inoue et al. Hepatology 2019 [10]	HBsAg	Serum	Lumipulse HBsAg-HQ
Pronier et al. J Clin Virol 2020 [16]	HBsAg	Serum	
Kuhns et al. Hepatology 2019 [11]	HBsAg	Serum	Abbott Architect

*Also found surrogate marker using serum HBcAb IgG titer > 4.4 associated with intrahepatic cccDNA

HBsAg hepatitis B surface antigen, *HBV* hepatitis B virus

A recent mathematical model estimated that 3.3% and 14% of OBI-infected blood donations (based on 20-mL vs 200-mL plasma samples, respectively) can transmit HBV; the limit of detection was approximately 18 copies/mL [21]. Additionally, a recent study showed a high rate of HBV transmission (29% of 31 recipients) due to transfusions from 3 donors with undetectable OBI [22•]. This study showed that levels as low as 16 copies/mL can transmit HBV; transmission was noted in recipients of greater plasma volume (red blood cells and fresh frozen plasma vs platelet transfusions). Additionally, recipients with HBsAb may have decreased risk of HBV transmission after blood transfusion.

Hemodialysis

The prevalence of OBI in dialysis patients has been reported as high as 58% [23]. Patients who receive hemodialysis are at high risk of blood-borne infections such as HBV due to a prolonged vascular access to shared dialysis machines, invasive procedures required for dialysis access, and anemia requiring occasional blood transfusions. Additionally, alterations in cellular and humoral immunity in patients with end-stage renal disease can result in suboptimal responses to HBV vaccination, increasing the risk of developing OBI [24]. Additional doses of vaccine or optimal vaccine formulations should be considered in those starting or receiving dialysis where there is an opportunity for protection with vaccination. Lastly, the presence of OBI in patients with end-stage renal disease who undergo renal transplantation increases the risk of HBV reactivation in the presence of immunosuppression. Providers should be cognizant of screening for OBI and vaccinating appropriately during earlier stages of chronic kidney disease when the immune response to vaccinations has not diminished.

Liver Transplantation

Transplantation of seropositive OBI organ donors (HBcAb+) puts HBV-susceptible recipients at risk of HBV transmission [25]. In particular, liver transplantation, compared to other solid organs, has the highest risk of HBV transmission given the persistence of donor liver total and cccDNA in OBI [26–29]. Transmissibility is greatest in HBsAb-negative recipients [28]. Both donor and recipient should be screened for HBV DNA using highly sensitive molecular assays to identify the presence of OBI given the risk of HBV reactivation with immunosuppression.

Identifying seronegative OBI in organ donors is highly unlikely without the use of highly sensitive assays for serum HBV DNA or an investigation for intrahepatic cccDNA. Therefore, HBV transmission and development of OBI in the recipient is still possible in cases where donors are considered high risk due to exposures for possible blood-borne infections. In recipients of high-risk donors, liver tests should be monitored carefully for early detection of OBI and HBV reactivation.

While the presence of HBsAb due to vaccination portends a lower risk of acquiring HBV, it does not eliminate HBV transmission. A case report of a HBV-vaccinated child transplanted from a donor who missed HBV serology evaluation resulted in the development of OBI related to a vaccine-escape HBV variant; it was later found that the donor was HBcAb-positive [30]. Chemoprophylaxis should be used in recipients of HBcAb-positive donors given the inevitable integration of episomal cccDNA in the liver allograft.

Mother to Child Transmission

Vaccination of HBV at birth has been a critical feat in decreasing HBV-related morbidity globally. However, it is not flawless as evidenced by the prevalence of OBI in newborns. In a study of 460 Taiwanese subjects, both children and adults, with isolated HBsAb positivity (due to a universal vaccination program) with negative HBsAg and negative HBcAb, 6% were positive for HBV DNA, indicating seropositive OBI. Sequence analysis revealed the presence of S gene mutation in 60% of cases, indicating the presence of vaccine-escape HBV variants [31]. A study from India showed that 64% of 222 babies had OBI during evaluation at 18 weeks and 42% at a median of 24 months [32]. Interestingly, some children lost their infection as they grew older with maturation of their immune system. Given this troubling clinical scenario, providers must not be complacent about the absence of OBI due to a history of vaccination and presence of HBsAb alone when approaching patients with liver-related morbidities.

Management

An overview of OBI and its clinical impact is shown in Fig. 2. Currently, there are no recommendations in treating patients with OBI alone. However, the strategy in treating and managing patients with OBI is primarily preventive given the risk of HBV reactivation. The American Association for the Study of Liver Diseases (AASLD) criteria for HBV reactivation include (1) a rise in HBV DNA compared to baseline and (2) reverse seroconversion from HBsAg negative to HBsAg positive in those with HBcAb positivity [33]. In certain clinical scenarios, chemoprophylaxis to prevent reactivation may be necessary.

Occult Hepatitis B and HIV

Individuals with HIV have higher rates of OBI [34]. In patients with concomitant HIV and OBI, providers should consider anti-HBV-directed antiretroviral therapy (ART)

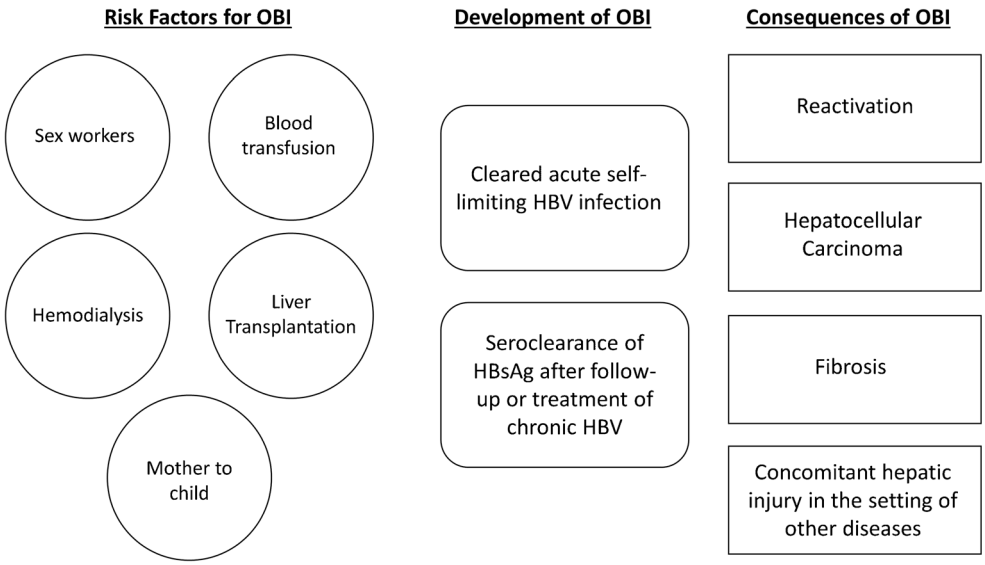

Fig. 2 Clinical impact of occult hepatitis B infection. Abbreviations: HBV = hepatitis B virus

regimens due to an increased risk of liver fibrogenesis and HBV reactivation. Proinflammatory and profibrogenic cytokine levels such as IL-8 and TNF-B1 have been detected in patients co-infected with HIV and OBI [35]. In a study of 86 patients naïve to ART for HIV, a higher prevalence of HBV reactivation was noted in those with OBI with positive serum HBV DNA (65% vs 25% in non-HBV viremic patients, $p < 0.005$) [36]. Additionally, the clinical manifestation of HIV with acquired immunodeficiency syndrome increases the risk of OBI and HBV reactivation due a heavily impaired immune system. The AASLD recommends that all patients with HIV be considered for testing for concomitant OBI with serum HBcAb [33]; the Infectious Diseases Society of America recommends that all patients with HIV be screened for HBsAg, HBsAb, and HBcAb [37]. Lastly, newer tenofovir-sparing ART regimens used to simplify treatment and increase adherence may increase the risk of OBI-related complications in HIV patients [38]. Therefore, providers must be cognizant of ART regimen changes in the context of OBI and potential risk of HBV reactivation.

Occult Hepatitis B and Hepatitis C

Similar to HIV, patients with hepatitis C virus (HCV) infection also have higher rates of concomitant OBI [1, 39]. Due to the presence of both HBV and HCV genomes in a single hepatocyte, HCV molecules interfere with HBV replication, resulting in low levels of HBV viremia [40]. Therefore, treating HCV has been associated with an increased risk of HBV reactivation in those with chronic HBV while the risk in those with OBI is low [41, 42]. The joint practice guidance from the AASLD and Infectious Diseases Society of America state there is insufficient data to provide clear guidance for the monitoring of HBV DNA among persons testing positive for HBcAb alone; however, note that the possibility of HBV reactivation should be considered in the event of an unexplained elevation in liver aminotransferase during and/or after completion of HCV therapy [43]. Self-limiting hepatitis has been reported in 2.4% of those with OBI receiving direct-acting antiviral therapy for HCV [44] in addition to a case report of fulminant hepatic failure resulting from HBV reactivation after HCV treatment with a NS3/4A protease inhibitor in a patient with OBI [45].

Occult Hepatitis B and Immunosuppressive Therapy

The use of chemotherapy and/or immunosuppressive therapy increases the risk of HBV reactivation in the setting of OBI, particularly with B cell–depleting agents (i.e., rituximab) [46]. Society guidelines now recommend chemoprophylaxis in most cases of patients with OBI planning to undergo chemotherapy and/or immunosuppressive therapy, depending on the regimen used [33, 47, 48]. A guideline summary from the American Gastroenterological Association Institute is shown in Supplementary Table 1 [49].

As previously mentioned, liver transplantation of HBcAb-positive donors increases the risk of OBI. Given the risk of HBV reactivation in the setting of immunosuppression, it is recommended that all HBV-susceptible recipients who receive HBcAb-positive donors be placed on lifelong nucleoside(tide) analogue inhibitor therapy to prevent HBV reactivation. Despite chemoprophylaxis, a majority of patients may develop OBI as evidenced by longitudinal analysis of serum, liver, and peripheral blood mononuclear cells showing detectable HBV DNA [50].

Occult Hepatitis B in Other Diseases

The interplay between OBI and the development of chronic liver disease is unclear due to the presence of other concomitant diseases. A meta-analysis of 14 studies evaluated 1503 subjects with and 2052 subjects without chronic liver disease and presence of OBI. With an odds ratio of 8.9 (95% CI: 4.1–19.5), OBI may be a significant factor in the development of chronic liver disease [51].

In a study of 226 obese patients undergoing bariatric surgery in Italy, liver tissue analysis showed that OBI was identified in 13% of cases; 83% of OBI cases had non-alcoholic steatohepatitis compared to 46% of non-OBI ($p < 0.05$) [52]. This study signified the role of OBI in disease progression in patients with fatty liver disease. Whether OBI alone can cause significant fibrosis development is unclear, and larger cohort studies are needed to evaluate for its effect on fibrogenesis.

Hepatitis D virus (HDV) infection is exclusively noted in the setting of HBV. Despite the lack of HBsAg, HDV RNA has been detected in select cases of OBI [53]. The effect of HDV pathogenicity in these cases on clinical outcomes is unknown.

Occult Hepatitis B and Hepatocellular Carcinoma

Currently, there are no recommendations for the screening of HCC in patients with OBI without advanced fibrosis, cirrhosis, or history of HCC. However, a recent review of OBI and HCC presented several studies that support the maintenance of pro-oncogenic properties of HBV in OBI. Pathophysiologically, this has been attributed to integration of HBV DNA into hepatocyte DNA, promoting effects of protein in malignant transformation and the development of necroinflammation contributing to carcinogenesis [54]. A meta-analysis of 16 studies, both retrospective and prospective, showed that subjects with OBI, including those with concomitant chronic HCV, cryptogenic liver disease, and chronic liver disease, had greater risk of HCC compared to non-infected subjects [55]. A prospective study followed a cohort of 82 patients with cirrhosis (negative for HBV and HCV) for a median follow-up of 5.8 years. Nine patients (11%) were identified as having OBI; 100% of OBI subjects developed HCC by the 10th year of follow-up compared to 18% in non-OBI subjects [56].

A recent study evaluated the involvement of OBI and HCC using integration analysis of HBV DNA [14••]. Integration analysis includes extraction of total DNA from liver tissue and evaluating intrahepatic HBV DNA by PCR analysis targeting HBV surface, precore, polymerase, and X regions; the Alu-PCR technique was used to identify HBV integrants. In this study, despite 91% being non-cirrhotic, there was a high prevalence of OBI and HCC (69%). Interestingly, this study postulated different pathophysiologic mechanisms of OBI depending on the virologic form of HBV DNA. The authors discuss the difference between cccDNA compared to integrated HBV DNA, with the former playing a role in HBV reactivation while the latter plays a role in HCC carcinogenesis.

As previously mentioned, those with OBI who achieved HBsAg seroclearance after chronic HBV have a reduced but persistent risk of HCC. In a study of 298 patients with HBsAg seroclearance, 2.4% of patients developed HCC; the risk was higher after the age of 50 years [5]. In a large cohort study of 20,263 subjects where 376 achieved HBsAg seroclearance, the risk of HCC was 0.6% at 8 years [57]. In contrast, the risk of HCC in OBI after spontaneous clearance of acute HBV is unknown.

Several published studies, both cross-sectional and prospective, have analyzed the development of HCC in those with OBI co-infected with HCV; controversy exists regarding the presence of OBI and its role in HCC development in the setting of underlying HCV [54]. In studies showing an association, it is unclear whether OBI is an independent risk factor or simply synergy in the manifestation of HCC in patients with underlying advanced fibrosis or cirrhosis. Recently, a study of 50 Egyptian patients with HCV-associated HCC showed a prevalence rate of 50% of OBI. The presence of OBI was associated with advanced histologic grading of HCC [58]. Understanding the natural history of OBI and HCC carcinogenesis is necessary to determine if HCC screening in OBI should be performed.

Conclusion

OBI remains an underrecognized clinical entity due to complexity in serologic and virologic detection methods. Monitoring patients with OBI in the setting of immunosuppressive therapy given the risk of HBV reactivation has been widely advocated for by professional societies. While still widely debated, recent studies have implicated OBI in liver-related outcomes, including chronic liver disease and HCC. In the future, improved diagnostics to determine the presence of HBsAg and/or HBV DNA will aid in better prevalence estimates of OBI. This data will be vital in shaping healthcare policy regarding screening, treatment, and evaluating for liver outcomes in patients with OBI. Nonetheless, in settings where HBV reactivation is a high possibility such as immunosuppressive therapy, liver transplantation, and HIV, providers must screen patients for OBI to determine the need for monitoring and/or chemoprophylaxis.

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Perumalswami—revised draft of manuscript, approved final draft submitted

Compliance with Ethical Standards

Conflict of Interest The authors do not have conflict of interest to declare.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

References

Papers of particular interest, published recently, have been highlighted as:
- • Of importance
- •• Of major importance

1.•• Raimondo G, Locarnini S, Pollicino T, Levrero M, Zoulim F, Lok AS. Update of the statements on biology and clinical impact of occult hepatitis B virus infection. J Hepatol. 2019;71:397–408. **This paper summarizes the most recent statement of the Taormina Workshop on OBI from 2018**.

2. Scotto G, Fazio V, Nicola C. Occult Hepatitis B Infection in recent immigrants to Italy: occult B infection in immigrants. J Community Health. 2020;45:357–62.

3. Urbani S, Fagnoni F, Missale G, Franchini M. The role of anti-core antibody response in the detection of occult hepatitis B virus infection. Clin Chem Lab Med. 2010;48:23–9.

4.• Caviglia GP, Abate ML, Tandoi F, et al. Quantitation of HBV cccDNA in anti-HBc-positive liver donors by droplet digital PCR: a new tool to detect occult infection. J Hepatol. 2018;69:301–7 **This study evaluates the use of droplet digital PCR to identify intrahepatic cccDNA in cases of positive HBcAb serology.**

5. Yuen MF, Wong DK, Fung J, et al. HBsAg seroclearance in chronic hepatitis B in Asian patients: replicative level and risk of hepatocellular carcinoma. Gastroenterology. 2008;135:1192–9.

6. Chaudhuri V, Tayal R, Nayak B, Acharya SK, Panda SK. Occult hepatitis B virus infection in chronic liver disease: full-length genome and analysis of mutant surface promoter. Gastroenterology. 2004;127:1356–71.

7. Vivekanandan P, Kannangai R, Ray SC, Thomas DL, Torbenson M. Comprehensive genetic and epigenetic analysis of occult hepatitis B from liver tissue samples. Clin Infect Dis. 2008;46:1227–36.

8. Raimondo G, Allain JP, Brunetto MR, Buendia MA, Chen DS, Colombo M, et al. Statements from the Taormina expert meeting on occult hepatitis B virus infection. J Hepatol. 2008;49:652–7.

9. Wang H, Wang M, Huang J, Xu R, Liao Q, Shan Z, et al. Novel hepatitis B virus surface antigen mutations associated with occult genotype B hepatitis B virus infection affect HBsAg detection. J Viral Hepat. 2020;27:915–21.

10. Inoue T, Shinkai N, Kusumoto S, et al. 653 clinical efficacy of a newly developed and fully automated high-sensitive hepatitis B surface antigen (HBsAg) assay for monitoring Hepatitis B virus reactivation. Hepatology. 2019;70:409A.

11. Kuhns M, Holzmayer V, McNamara AL, et al. 663 molecular and serological characterization of hepatitis B early acute and occult specimens incrementally detected by a new increased sensitivity HBsAg assay. Hepatology. 2019;70:415A.

12. Akram A, Islam SMR, Munshi SU, Tabassum S. Detection of hepatitis B virus DNA among chronic and potential occult HBV patients in resource-limited settings by loop-mediated isothermal amplification assay. J Viral Hepat. 2018;25:1306–11.

13. Saitta C, Tripodi G, Barbera A, Bertuccio A, Smedile A, Ciancio A, et al. Hepatitis B virus (HBV) DNA integration in patients with occult HBV infection and hepatocellular carcinoma. Liver Int. 2015;35:2311–7.

14.•• Wong DK, Cheng SCY, Mak LL, et al. Among patients with undetectable hepatitis B surface antigen and hepatocellular carcinoma, a high proportion has integration of HBV DNA into hepatocyte DNA and no cirrhosis. Clin Gastroenterol Hepatol. 2020;18:449–56. **This study identified HBV DNA integration as a possible pathophysiologic mechanism for OBI and HCC.**

15. Liu Y, Zeng W, Xi J, Liu H, Liao H, Yu G, et al. Over-gap PCR amplification to identify presence of replication-competent HBV DNA from integrated HBV DNA: an updated occult HBV infection definition. J Hepatol. 2019;70:557–9.

16. Pronier C, Candotti D, Boizeau L, Bomo J, Laperche S, Thibault V. The contribution of more sensitive hepatitis B surface antigen assays to detecting and monitoring hepatitis B infection. J Clin Virol. 2020;129:104507.

17. Pinarbasi B, Onel D, Cosan F, Akyuz F, Dirlik N, Cakaloglu Y, et al. Prevalence and virological features of occult hepatitis B virus infection in female sex workers who work uncontrolled in Turkey. Liver Int. 2009;29:227–30.

18. Jepkemei KB, Ochwoto M, Swidinsky K, Day J, Gebrebrhan H, McKinnon LR, et al. Characterization of occult hepatitis B in high-risk populations in Kenya. PLoS One. 2020;15:e0233727.

19. Yuen MF, Wong DK, Lee CK, et al. Transmissibility of hepatitis B virus (HBV) infection through blood transfusion from blood donors with occult HBV infection. Clin Infect Dis. 2011;52:624–32.

20. González R, Torres P, Castro E, et al. Efficacy of hepatitis B virus (HBV) DNA screening and characterization of acute and occult HBV infections among blood donors from Madrid, Spain. Transfusion. 2010;50:221–30.

21. Weusten J, van Drimmelen H, Vermeulen M, Lelie N. A mathematical model for estimating residual transmission risk of occult hepatitis B virus infection with different blood safety scenarios. Transfusion. 2017;57:841–9.

22.• Candotti D, Assennato SM, Laperche S, Allain JP, Levicnik-Stezinar S. Multiple HBV transfusion transmissions from undetected occult infections: revising the minimal infectious dose. Gut. 2019;68:313–21 **This study conveys the possibility of OBI transmission with a minimal infectious dose of 100 to 16 copies (or 3 IU) in blood products, primarily red blood cells and fresh frozen plasma.**

23. Fontenele AM, Filho NS, Ferreira AS. Occult hepatitis B in patients on hemodialysis: a review. Ann Hepatol. 2013;12:527–31.

24. Soi V, Soman S. Preventing hepatitis B in the dialysis unit. Adv Chronic Kidney Dis. 2019;26:179–84.

25. De Feo TM, Poli F, Mozzi F, Moretti MP, Scalamogna M. Risk of transmission of hepatitis B virus from anti-HBC positive cadaveric organ donors: a collaborative study. Transplant Proc. 2005;37:1238–9.

26. Dickson RC, Everhart JE, Lake JR, Wei Y, Seaberg EC, Wiesner RH, et al. Transmission of hepatitis B by transplantation of livers from donors positive for antibody to hepatitis B core antigen. The National Institute of Diabetes and Digestive and Kidney Diseases

Liver Transplantation Database. Gastroenterology. 1997;113: 1668–74.
27. Prieto M, Gómez MD, Berenguer M, Córdoba J, Rayón JM, Pastor M, et al. De novo hepatitis B after liver transplantation from hepatitis B core antibody-positive donors in an area with high prevalence of anti-HBc positivity in the donor population. Liver Transpl. 2001;7:51–8.
28. Cholongitas E, Papatheodoridis GV, Burroughs AK. Liver grafts from anti-hepatitis B core positive donors: a systematic review. J Hepatol. 2010;52:272–9.
29. Hussain M, Soldevila-Pico C, Emre S, Luketic V, Lok AS. Presence of intrahepatic (total and ccc) HBV DNA is not predictive of HBV recurrence after liver transplantation. Liver Transpl. 2007;13:1137–44.
30. Salpini R, Pietrobattista A, Piermatteo L, Basso MS, Bellocchi MC, Liccardo D, et al. Establishment of a seronegative occult infection with an active hepatitis B virus reservoir enriched of vaccine escape mutations in a vaccinated infant after liver transplantation. J Infect Dis. 2019;220:1935–9.
31. Lai MW, Lin TY, Liang KH, Lin WR, Yeh CT. Hepatitis B viremia in completely immunized individuals negative for anti-hepatitis B core antibody. Medicine (Baltimore). 2016;95:e5625.
32. Pande C, Sarin SK, Patra S, Kumar A, Mishra S, Srivastava S, et al. Hepatitis B vaccination with or without hepatitis B immunoglobulin at birth to babies born of HBsAg-positive mothers prevents overt HBV transmission but may not prevent occult HBV infection in babies: a randomized controlled trial. J Viral Hepat. 2013;20:801–10.
33. Terrault NA, Lok ASF, McMahon BJ, et al. Update on prevention, diagnosis, and treatment of chronic hepatitis B: AASLD 2018 hepatitis B guidance. Hepatology. 2018;67:1560–99.
34. Lo Re V 3rd, Frank I, Gross R, et al. Prevalence, risk factors, and outcomes for occult hepatitis B virus infection among HIV-infected patients. J Acquir Immune Defic Syndr. 2007;44:315–20.
35. Martin CM, Welge JA, Shire NJ, Shata MT, Sherman KE, Blackard JT. Cytokine expression during chronic versus occult hepatitis B virus infection in HIV co-infected individuals. Cytokine. 2009;47: 194–8.
36. Filippini P, Coppola N, Pisapia R, Scolastico C, Marrocco C, Zaccariello A, et al. Impact of occult hepatitis B virus infection in HIV patients naive for antiretroviral therapy. Aids. 2006;20:1253–60.
37. Aberg JA, Gallant JE, Ghanem KG, Emmanuel P, Zingman BS, Horberg MA. Primary care guidelines for the management of persons infected with HIV: 2013 update by the HIV Medicine Association of the Infectious Diseases Society of America. Clin Infect Dis. 2014;58:1–10.
38. Soriano V, Aguilera A, Gonzalez R, Gomez-Gallego F, Barea L, Treviño M, et al. Occult hepatitis B and HIV infection. Eur J Gastroenterol Hepatol. 2019;31:1403–7.
39. Chen HY, Su TH, Tseng TC, Yang WT, Chen TC, Chen PJ, et al. Impact of occult hepatitis B on the clinical outcomes of patients with chronic hepatitis C virus infection: a 10-year follow-up. J Formos Med Assoc. 2017;116:697–704.
40. Rodríguez-Iñigo E, Bartolomé J, Ortiz-Movilla N, et al. Hepatitis C virus (HCV) and hepatitis B virus (HBV) can coinfect the same hepatocyte in the liver of patients with chronic HCV and occult HBV infection. J Virol. 2005;79:15578–81.
41. Liu CJ, Chuang WL, Sheen IS, Wang HY, Chen CY, Tseng KC, et al. Efficacy of ledipasvir and sofosbuvir treatment of HCV infection in patients coinfected with HBV. Gastroenterology. 2018;154: 989–97.
42. Mücke MM, Backus LI, Mücke VT, Coppola N, Preda CM, Yeh ML, et al. Hepatitis B virus reactivation during direct-acting antiviral therapy for hepatitis C: a systematic review and meta-analysis. Lancet Gastroenterol Hepatol. 2018;3:172–80.
43. Ghany MG, Morgan TR. Hepatitis C guidance 2019 update: American Association for the Study of Liver Diseases-Infectious Diseases Society of America Recommendations for Testing, Managing, and Treating Hepatitis C Virus Infection. Hepatology. 2020;71:686–721.
44. Wang C, Ji D, Chen J, Shao Q, Li B, Liu J, et al. Hepatitis due to reactivation of hepatitis B virus in endemic areas among patients with hepatitis C treated with direct-acting antiviral agents. Clin Gastroenterol Hepatol. 2017;15:132–6.
45. Ende AR, Kim NH, Yeh MM, Harper J, Landis CS. Fulminant hepatitis B reactivation leading to liver transplantation in a patient with chronic hepatitis C treated with simeprevir and sofosbuvir: a case report. J Med Case Rep. 2015;9:164.
46. Seto WK, Chan TS, Hwang YY, et al. Hepatitis B reactivation in patients with previous hepatitis B virus exposure undergoing rituximab-containing chemotherapy for lymphoma: a prospective study. J Clin Oncol. 2014;32:3736–43.
47. Reddy KR, Beavers KL, Hammond SP, Lim JK, Falck-Ytter YT. American Gastroenterological Association Institute guideline on the prevention and treatment of hepatitis B virus reactivation during immunosuppressive drug therapy. Gastroenterology. 2015;148: 215–9 quiz e16–7.
48. EASL. 2017 Clinical Practice Guidelines on the management of hepatitis B virus infection. J Hepatol. 2017;67:370–98.
49. Perrillo RP, Gish R, Falck-Ytter YT. American Gastroenterological Association Institute technical review on prevention and treatment of hepatitis B virus reactivation during immunosuppressive drug therapy. Gastroenterology. 2015;148:221–44.e3.
50. Coffin CS, Mulrooney-Cousins PM, van Marle G, Roberts JP, Michalak TI, Terrault NA. Hepatitis B virus quasispecies in hepatic and extrahepatic viral reservoirs in liver transplant recipients on prophylactic therapy. Liver Transpl. 2011;17:955–62.
51. Covolo L, Pollicino T, Raimondo G, Donato F. Occult hepatitis B virus and the risk for chronic liver disease: a meta-analysis. Dig Liver Dis. 2013;45:238–44.
52. Raimondo G, Saitta C, Lombardo D, et al. Occult hepatitis B virus infection predicts non-alcoholic steatohepatitis in severely obese individuals from Italy. Liver Int. 2020;40(7):1601.
53. Delfino CM, Eirin ME, Berini C, et al. HDAg-L variants in covert hepatitis D and HBV occult infection among Amerindians of Argentina: new insights. J Clin Virol. 2012;54:223–8.
54. Mak LY, Ka-Ho Wong D, Pollicino T, Raimondo G, Hollinger FB, Yuen MF. Occult hepatitis B infection and hepatocellular carcinoma: epidemiology, virology, hepatocarcinogenesis and clinical significance. J Hepatol. 2020;73:952–64.
55. Shi Y, Wu YH, Wu W, Zhang WJ, Yang J, Chen Z. Association between occult hepatitis B infection and the risk of hepatocellular carcinoma: a meta-analysis. Liver Int. 2012;32:231–40.
56. Ikeda K, Kobayashi M, Someya T, Saitoh S, Hosaka T, Akuta N, et al. Occult hepatitis B virus infection increases hepatocellular carcinogenesis by eight times in patients with non-B, non-C liver cirrhosis: a cohort study. J Viral Hepat. 2009;16:437–43.
57. Yip TC, Wong GL, Chan HL, et al. HBsAg seroclearance further reduces hepatocellular carcinoma risk after complete viral suppression with nucleos(t)ide analogues. J Hepatol. 2019;70:361–70.
58. El-Maksoud MA, Habeeb MR, Ghazy HF, et al. Clinicopathological study of occult hepatitis B virus infection in hepatitis C virus-associated hepatocellular carcinoma. Eur J Gastroenterol Hepatol. 2019;31:716–22.